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GRANT NUMBER: DAMD17-94-J-4077

TITLE: A Comprehensive Repository of Normal and Tumor Human
Breast Tissue and Cells

PRINCIPAL INVESTIGATOR: Jerry W. Shay, Ph.D.

CONTRACTING ORGANIZATION: University of Texas at Dallas
Dallas, Texas 75235-8593

REPORT DATE: July 1996

TYPE OF REPORT: Annual

PREPARED FOR: Commander
U.S. Army Medical Research and Materiel Command
Fort Detrick, Frederick, Maryland 21702-5012

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19960919 004

DTIC QUALITY INSPECTED 1

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.

1. AGENCY USE ONLY (Leave blank)		2. REPORT DATE July 1996		3. REPORT TYPE AND DATES COVERED Annual (1 Jul 95 - 30 Jun 96)	
4. TITLE AND SUBTITLE A Comprehensive Repository of Normal and Tumor Human Breast Tissue and Cells				5. FUNDING NUMBERS DAMD17-94-J-4077	
6. AUTHOR(S) Jerry W. Shay, Ph.D.					
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Texas at Dallas Dallas, Texas 75235-8593				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) Commander U.S. Army Medical Research and Materiel Command Fort Detrick, MD 21702-5012				10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES					
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited				12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200) The development of breast cancer is likely to be a multi-step progressive process with several heritable alterations accumulated during the evolution to malignancy. In order to have surrogate endpoint markers to provide a means for early intervention, it is important to define the specific molecular alterations at each stage of this process. The study of such alterations should be greatly facilitated by having not only cells derived from breast tumors but also corresponding non-malignant breast cells of stromal and epithelial origin. We have successfully established a unique repository which contains cryopreserved and cultured cells from human breast tumor tissues, non-malignant breast epithelial and stromal cells and peripheral blood mononuclear cells.					
14. SUBJECT TERMS Breast Cancer , breast tumor cell lines, epithelial/stromal cells				15. NUMBER OF PAGES 9	
				16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited		

FOREWORD

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A Comprehensive Repository of Normal and Tumor Human Breast Tissues and Cells

Introduction

The principal objectives of the program are to:

- 1) obtain and cryopreserve from breast cancer patients, peripheral blood mononuclear cells, tumor tissue, and non-malignant adjacent breast tissue;
- 2) prepare and cryopreserve breast tissue organoids from which both epithelial and stromal cells can be cultured;
- 3) establish breast tumor cell lines from patients with primary breast carcinoma;
- 4) establish and cryopreserve EBV-transformed B-lymphoblastoid cells as a source of constitutional DNA;
- 5) include samples from women with familial breast cancer and with non-invasive breast cancers;
- 6) collect patient demographic, family, clinical and pathological data;
- 7) maintain computerized records of all data, materials accessioned, and cell characterization;
- 8) publicize information about the repository and to make its resources readily available to the scientific community with minimal restrictions.

The samples will be characterized for DNA ploidy, karyotype, progesterone/estrogen receptors, BRST-1, BRST-2, CEA, cytokeratins, MFGMA, p53 mutations and telomerase activity. The repository will provide researchers with a comprehensive and unique source of frozen tissue and self-replicating tumor cells and corresponding epithelial and stromal breast cells as well as constitutional DNA, along with relevant clinical and demographic information.

Body

Task 1. Obtain Normal and Tumor Surgical Specimens

In July 1994 we initiated a repository for multiple areas of breast cancer research. It was an ambitious project, and during the first year of the parent grant we obtained and cryopreserved 84 tissue samples. Of these samples we established and cryopreserved a total of five human breast tumor cells lines. During the second year of the parent grant we obtained and cryopreserved 55 additional tissue samples. Efforts were undertaken to obtain early and premalignant breast tissue samples and during the second year of the parent grant we obtained one ductal carcinoma in situ, three lobular carcinoma in situ, 20 fibroadenomas, and 17 other benign conditions.

Prior to the initiation of our breast tumor and cell repository 85 breast cancer

specimens were accessioned. These consisted of 62 primary breast cancers and 23 metastatic lesions. When available primary tumor tissue, adjacent non-malignant tissue, and cryopreserved peripheral blood mononuclear cells were preserved in each case. During the first two years of the parent breast tumor and cell repository grant, we have obtained and cryopreserved approximately 139 samples so that at the present time we have accessioned a total of 224 individual breast specimens. Thus most of our effort during the first two years has been to accession samples and establish the cell lines. We have characterized all our primary tumors for the presence of telomerase activity. The development of an amplification protocol to measure telomerase activity assay has been successful and widely acknowledged as a major achievement in cancer research (see references).

Task 2. Culture Organoids from "Normal" Breast Tissue Samples and Separate Epithelial from Stromal Cells

We have continued to be successful in culturing and cryopreserving breast epithelial and stromal cell cultures. During the first two years a total of 23 human breast epithelial and 25 stromal cell strains have been cryopreserved. In addition, we have 50 additional organoid cultures frozen which have not been established into epithelial and stromal strains (see below). One of the epithelial cell cultures obtained from a patient with Li-Fraumeni syndrome spontaneously immortalized in cell culture (see references).

Task 3. Characterize Breast Epithelial and Stromal Cells

Due to limited manpower, we have elected to only characterize those epithelial and stromal cells in which tumor cell lines are established. Since it requires at least 4-6 months of culture to be confident that a primary tumor is successfully established, we generally make breast tissue organoids and in some instances primary cultures and then cryopreserve them until such time as the tumor cell data are obtained. As is detailed in task 4 we now have matched tumor derived cell lines and normal epithelial and stromal cells from three of our accessioned specimens. We are currently characterizing these strains, scaling them up and freezing back early passages for future distribution from the repository.

Task 4. Establish Breast Tumor Cell Lines from Primary Breast Carcinoma

We recognized at the onset that establishing breast tumor cell lines would be the rate limiting component to the success of the repository. At the end of the first year of the parent grant we had clearly established one additional breast tumor cell line (for a total of 5 new breast tumor cell lines). During the second year we made a special effort to initiate and obtain additional human breast tumor cell lines. We were successful in establishing 8 additional lines for a total of 13 lines that are currently in the repository. These new human breast tumor cell lines were almost all derived from primary invasive ductal breast carcinomas and are currently being characterized for DNA ploidy, karyotype, progesterone/estrogen receptors, BRST-1, BRST-2, CEA, cytokeratins, MFGMA, p53 mutations and telomerase activity. We hope to finish characterizing these lines in the next six months, to write a manuscript on their characterization and to

provide them to the ATCC for distribution to the scientific community. We were also very fortunate to have established normal epithelial and stromal cell strains from three of these new breast tumor cell lines (see task 3 above).

Task 5. Establish EBV-transformed B-lymphoblastoid Cell Lines

We have cryopreserved peripheral blood mononuclear cells from all patients from whom we obtained permission but decided that we would transform only those samples with EBV when we had preliminary evidence that the tumor lines were successfully established and cryopreserved. Of the 13 tumor cell lines that we have established, we have peripheral blood mononuclear cells frozen from 8 of the 13. Of these all 8 have been EBV-transformed and established as lines for a source of constitutional DNA. In addition, one of these EBV-transformed B-lymphoblastoid cell lines has accompanying normal breast and stromal cell strains and a tumor derived cell line. This is a unique combination of materials from one individual and will be a valuable asset for breast cancer research.

Task 6. Maintain a Computerized Database

All entries are currently made on a Macintosh computer in the co-investigator's laboratory (Dr. Gazdar). Patient demographic information, and relevant clinical and family data are collected and entered onto a computerized relational database written in the Fourth Dimension software program with access by password. A database has been appropriately modified by Mr. David Wheeless, Computer Specialist, an employee of the Simmons Cancer Center at the University of Texas Southwestern Medical Center. Only Drs. Shay, Gazdar, and personnel with a need to know have access to patient identification. Informed consents and other hard copies of patient data are stored in locked, limited access cabinets. Responsibility for computer entries are given to a single person (with the confirmation of correct entry given to a second person). Backup of the data base is made weekly onto a tape drive (automatic via network). All samples are coded, divided and maintained in both liquid nitrogen and -150°C freezers (with automatic alarms). The freezers are located in separate buildings. Only designated personnel are able to access the repository.

Task 7. Making Samples Available to Breast Cancer and Other Researchers

Even though we have not advertized during the first two years, 39 individuals have obtained tissues and cells from our repository.

Task 8. Maintenance of Cell Repository and Backup

At present all samples are maintained in both Dr. Gazdar's and Dr. Shay's laboratories. During this review period, we have initiated efforts to contact existing breast tissue banks to attempt to coordinate data base interconnections. During the present review period we have contacted Drs. Tom Frank and Steve Ethier at the University of Michigan Cancer Center who have just established a web site for their breast tumor repository (<http://www.cancer.med.umich.edu/>). In addition, Dr. Martha Stampfer (Lawrence Berkeley Laboratory, California) has also just established a home page on

human mammary epithelial cells (<http://www.lbl.gov/~mrgs>) and we have contacted her. It is our intention during the next review period to establish our own web site and if possible to link our site with the Michigan and California site. We are in discussions with both the Michigan and California groups about establishing links between our web sites and establishing an electronic bulletin board that would include some of the following: availability of cells and tissues; posted information from others who have resources to share (cells, antibodies, cDNAs etc); posted questions and requests from others on the network; and indexed information about latest experimental results/abstracts they would like to share. In addition, at the NCI there is a Cooperative Breast Cancer Tissue Repository directed by Roger L. Aamodt, Ph.D. This registry provides tissue sections from formalin-fixed, paraffin-embedded primary breast tumors. This registry can provide clinical and outcome data, including demographic data, diagnosis, extent of disease, treatment, follow-up, recurrence, survival and vital statistic. These formalin-fixed materials should complement our frozen repository materials, and we will contact Dr. Aamodt about interconnecting our efforts.

Task 9. Future Stable Monetary Support for Repository

Since we have only completed two of the four years of the grant, we have decided to delay until the end of third or perhaps the beginning of the fourth year pursuing additional long term support for the repository.

Conclusions

We have had a very successful effort in the first two years of the repository. Initially we had to recruit and train a new research assistant and establish lines of communication for successfully obtaining and distributing samples. We were somewhat disappointed in the first year that we had not clearly established more tumor cells lines, but during the second year we have had considerably more success. One of our biggest successes was the development of an improved telomerase activity assay which we used to characterize 140 human breast tumors, 55 adjacent noncancerous breast tissue specimens, and other noncancerous lesions including 20 fibroadenomas and 17 fibrocystic disease specimens (see references). In addition, we successfully established a breast epithelial cell line from a patient with Li-Fraumeni syndrome (one of the first spontaneously immortalized human breast epithelial lines reported). Finally, and perhaps most importantly for future breast cancer research, we have successfully established 13 new human breast tumor cell lines and from three of these we also have non cancerous human breast epithelial and stromal cell strains. These new reagents should facilitate progress in breast cancer research in the future.

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